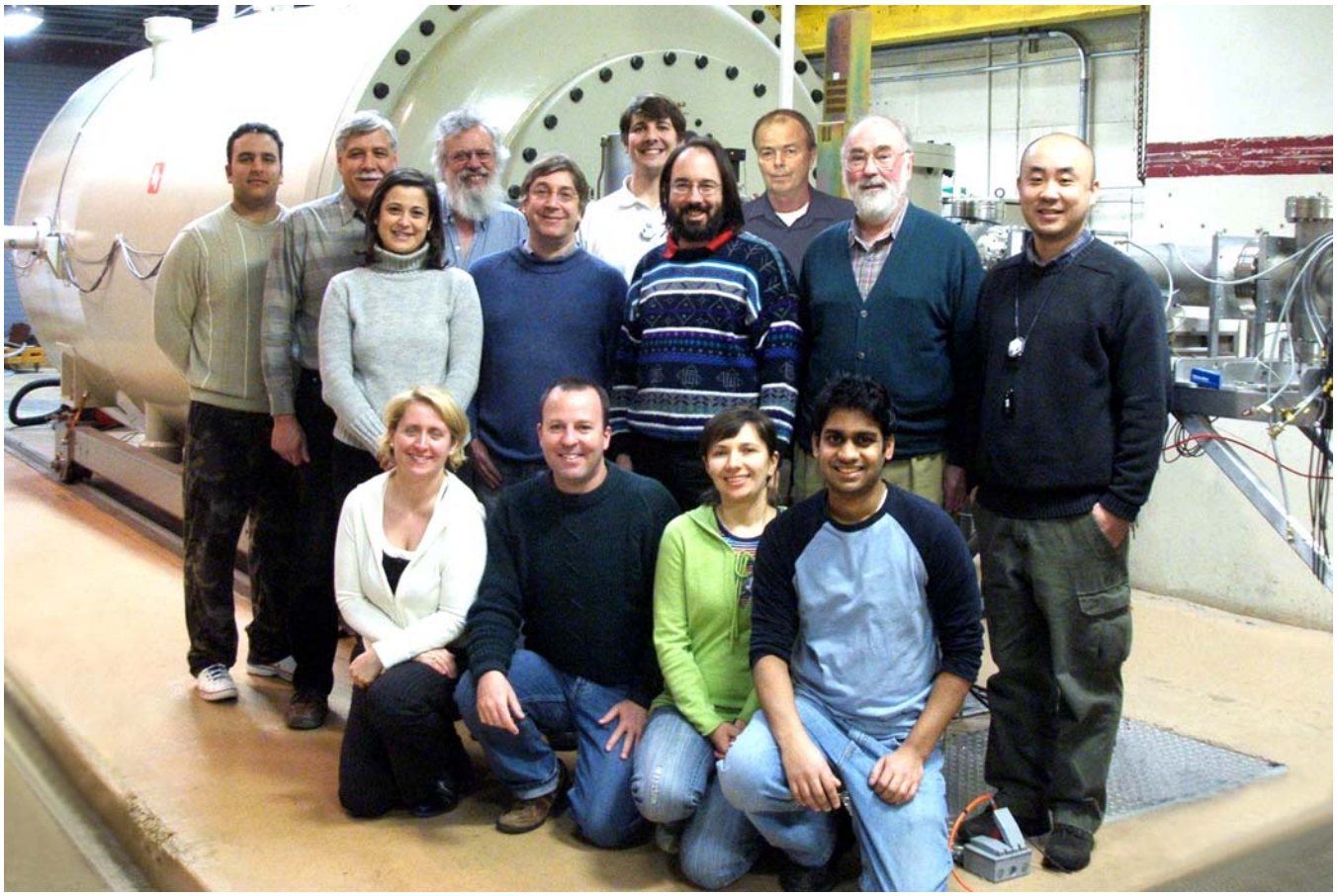
RARAF – Table of Contents

RARAF PROFESSIONAL STAFF



RARAF Staff *(l–r)*: *Front row*: Helen Turner, Alan Bigelow, Sasha Lyulko and Bharat Patel; *2nd* row: Antonella Bertucci, David Brenner, Guy Garty, Charles Geard and Yanping Xu; *3rd row*: Abel Bencosme, Stephen Marino, Gerhard Randers-Pehrson, Andrew Harken and Gary Johnson. Not shown: Brian Ponnaiya, Kenichi Tanaka and Gloria Jenkins-Baker.

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The Radiological Research Accelerator Facility

AN NIH-SUPPORTED RESOURCE CENTER – WWW.RARAF.ORG

Director: David J. Brenner, Ph.D., D.Sc.
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Introduction

The construction activity of the past two years is behind us. The Singletron, which replaced our aged Van de Graaff, is now over two years old and generally running well. The third floor laboratories are essentially complete. All the new third floor labs are in use and half the desks are presently occupied.

Research using RARAF

The main focus of the biological experiments at RARAF for the past several years has been the "bystander" effect, in which cells that are not irradiated show a response to radiation when in close contact with or even only in the presence of irradiated cells. Every biology experiment run this year examined this effect. The emphasis of the present experiments is to determine the mechanism(s) by which the effect is transmitted and whether the mechanisms are different for direct gap junction communication through cell membrane contact and indirect, long-range communication through the cell media. Both the microbeam and the track segment facilities continue to be utilized in various investigations of this phenomenon. The single-particle Microbeam Facility provides precise control of the number and location of particles so that irradiated and bystander cells may be distinguished but is somewhat limited in the number of cells that can be irradiated. The Track Segment Facility provides broad beam irradiation that has a random pattern of charged particles but allows large numbers of cells to be irradiated and multiple users in a single day.

Two special types of track segment dishes are being used to investigate the bystander effect using the Track Segment Facility: double-sided dishes and "strip" dishes. Double-sided dishes have thin (6-μm) Mylar foils glued on both sides of a stainless steel ring, 1cm apart, with cells plated on the inside surfaces of both foils. The interior is completely filled with medium. This type of dish is used for investigation of the non-contact, long-range bystander effect since the cells on the two surfaces are not in direct contact, can only communicate through the culture medium, and only the cells on one surface are irradiated. "Strip" dishes consist of a stainless steel ring with thin (6-μm) Mylar foil glued to one side in which a second dish is inserted. The Mylar foil glued to the inner dish has alternate strips of the Mylar removed. Cells are plated over the combined surface and are in contact. The Mylar on the inner dish is thick enough (38μm) to stop the charged particles (^{4}He ions) and the cells plated on it are not irradiated. These dishes are used for bystander experiments involving cell-to-cell communication.

Interest in irradiation of 3-D systems continued this past year, with tissue samples irradiated using either helium ions or protons. Imaging systems for the Microbeam Facility are being developed to enable observation and targeting of cells that are not in monolayers; in the interim, cultured human tissue samples are being irradiated using the Track Segment Facility. The tissue samples are on membranes on the end of cylindrical plastic holders. Plastic discs have been constructed that fit in the dish openings in the irradiation wheel and have small holes to provide precise alignment of the feet that are around the bottom edges of the tissue holders. A hole in the middle of each disc is fitted with two stainless steel half-discs that have a precise .001inch (25μm) space between them. The tissue membrane is in contact with the stainless steel, which is thick enough to stop the charged particles. This provides a narrow line of irradiation across the center of the entire sample. The tissue samples are later sectioned, either parallel or crosswise to the line of irradiation, to observe bystander effects as a function of distance from the line of irradiation.

The experiments performed at RARAF from January 1 through December 31, 2007 and the number of days each was run in this period are listed in Table 1. Fractional days are assigned when experimental time is shared among several users (e.g., track segment experiments) or experiments run for more or less than a shift. Use of the accelerator for experiments was 49% of the regularly scheduled time (40 hours per week), 15% lower than last year (which was the highest use we have attained at Nevis Labs) but about average for the last 5 years. Ten different experiments were run during this period. Six experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH), the National Aeronautics and Space Administration (NASA), and the Department of Energy (DoE). Four experiments were performed by outside users, supported by grants and awards from the NIH, the NSF, and the National Natural Science Foundation of China (NSFC). Brief descriptions of these experiments follow.

Gerhard Randers-Pehrson, Alan Bigelow and Yanping Xu of the CRR continued development of a method to detect explosives in baggage (Exp. 82). They have been assisted this year by Kenichi Tanaka, a visiting scientist from Hiroshima University. The detection system is based on resonant elastic scattering of 0.43 MeV neutrons by nitrogen and oxygen, which are present in higher percentages in most explosive materials. Measurements of the neutron transmission through sample materials are made using neutrons produced in a very thin target by the ^{7}Li(p,n) reaction. A high voltage is applied to the target and scanned slowly up and down across the proton energy required to produce neutrons

Table 1. Experiments Run at RARAF, January 1 - December 31, 2007

Exp. No.	Experimenter	Institution	Exp. Type	Experiment Title	No. Days Run
82	G Randers-Pehrson A. Bigelow Y. Xu	CRR	Physics	Detection of explosives	9.2
103	B. Hu C. R. Geard	CRR	Biology	Damage induction and characterization in known hit versus non-hit human cells	19.0
106	B. Ponnaiya C. R. Geard	CRR	Biology	Track segment α-particles, cell co-cultures and the bystander effect	0.5
110	H. Zhou Y-C. Lien M. Hong T. K. Hei	CRR	Biology	Identification of molecular signals of α-particle-induced bystander mutagenesis	31.1
112	Y. Horowitz A. Horowitz (S. A. Marino)	Ben Gurion Univ., Nuclear Research Ctr., Beersheva	Physics	HCP and neutron irradiation of LiF:Mg, Ti TLD chips to determine 5a/5 intensities and characterization of 5a peak as a Q/RBE nanodosimeter	9.5
123	E. Aprile	Columbia Univ., Astrophysics	Physics	Calibration of a liquid Xenon detector for weakly interacting massive particle (WIMPs)	29.1
133	S. Ghandhi J. Ahn, S. Amundson	CRR	Biology	Bystander effects in primary cells	6.4
136	S. Paul A. Mezentsev S. Amundson	CRR	Biology	Bystander effects in 3D tissues	6.2
138	E. Azzam J. Santos O. Kovalenko	NJSMD	Biology	Investigation of the effect of mtDNA damage on apoptosis in hTERT cells	1.0
140	L. Han (T. K. Hei)	Shanghai Medical University	Biology	Study of the mechanism of radiation-induced inactivation of the FHIT gene by single cell microbeam irradiation	10.6

Note: Names in parentheses are members of the CRR who collaborated with outside experimenters.

at the resonance energy. The neutron transmission can then be measured over the range of 20 keV under identical target and focusing conditions to observe the ratio of transmission at the resonance relative to off the resonance energy. They are presently working on a new target design to extend the life of the thin lithium layer used to produce the neutrons. Dr. Tanaka has worked on the design of a liquid nitrogen cold trap (Fig. 1) having a passage for the proton beam. The liquid nitrogen trap is extremely effective in removing water vapor from the system, which is a major cause of target deterioration. He has also been doing Monte Carlo neutron calculations using the MCNP code to assess the ability of the detection system to distinguish explosives from ordinary materials.

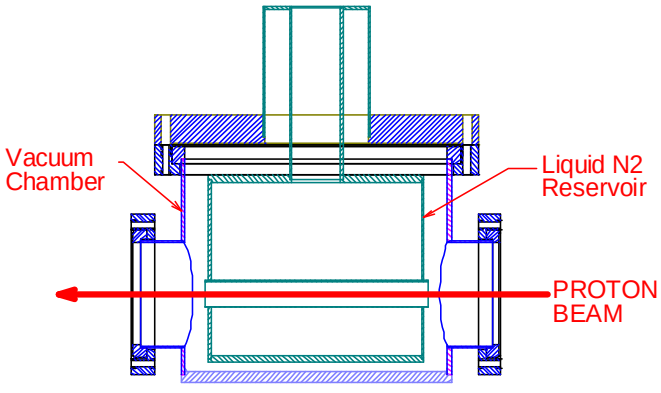

Fig. 1. Design of the liquid nitrogen trap for the oxygen/nitrogen resonance explosives detector.

Studies of the bystander effect, examining the relationship between the radiation-induced bystander response and genomic instability (Exp. 103), were continued by Burong Hu and Charles Geard of the CRR. Normal human lung fibroblasts were cultured in double-sided Mylar dishes (see above) and one side was irradiated with 0.1 to 5Gy of ^{4}He ions using the Track Segment Facility. The range of the helium ions is very much shorter than the space between the two Mylar layers so that the cells on the other side of the dish were then bystanders, which could only be influenced by signal transfer through the medium. For microbeam studies, 20% of the nuclei of nearly confluent (in contact) fibroblasts were irradiated with 30 ^{4}He ions each, which ensures that only non-hit bystander cells can survive over many cell generations. In both scenarios cells were harvested at 3h and 24h post-irradiation and after 5, 10, 15, 20 and 25 population doublings. Elevated levels of chromosomal damage in bystander cells were observed after G2-PCC, reflecting signal

transfer from irradiated cells, while elevated levels of chromosomal changes at later times as recorded by mFISH indicate genomic instability. Emphasis this year has been on intra-chromosomal changes in chromosome 11 in bystander cells and cells irradiated only in the cytoplasm.

Another study investigating the bystander effect was continued by Brian Ponnaiya and Charles Geard of the CRR (Exp. 106). The Track Segment Facility was used for broad-beam charged particle irradiations to examine genomic instability in irradiated and bystander htert immortalized human bronchial epithelial cells (HBEC-3kt, obtained from J. Shay). These cells were cultured on standard single-sided Mylar dishes and irradiated with half the dish covered by a thin metal shield. Cells on the non-covered portion of the dishes were irradiated with 0.5 and 1 Gy of ^{4}He ions, while cells on the covered portions of the dishes were bystander cells. Irradiated and bystander populations from each dish were separated and set up in culture. At various times post irradiation (7-28 days) G2-PCCs were prepared from each culture using Calyculin A. The chromosomes were analyzed by both Giemsa staining (for gross chromosomal aberrations) and mFISH for more subtle alterations (e.g. Translocations). Giemsa staining as well as mFISH revealed that both irradiated and bystander populations had elevated yields of chromosomal changes at 7 and 14 days post irradiation.

Hongning Zhou, Yu-Chin Lien, Mei Hong and Tom Hei of the CRR continued to use the Track Segment Facility and the single-particle Microbeam Facility to try to identify the cell-to-cell signaling transduction pathways involved in radiation-induced bystander mutagenesis (Exp. 110). Using the charged particle microbeam, they found that mitochondrial DNA-depleted human skin fibroblasts (ρ^0) showed a higher bystander mutagenic response in confluent monolayers when a fraction of the same population was irradiated with a lethal dose of alpha particles in the nucleus compared to their parental, mitochondria-functional cells (ρ^+). Using mixed cultures of ρ^0 and ρ^+ cells and targeting only one population of cells with a lethal dose, decreased bystander mutagenesis was uniformly found in non-irradiated bystander cells of both cell types, indicating that signals from one cell type can modulate expression of the bystander response in another cell type. In addition, they found that Bay 11-7082, a pharmacological inhibitor of nuclear factor-κB (NF-κB) activation, and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO), a scavenger of nitric oxide (NO), significantly decreased the mutation frequency in both bystander ρ^0 and ρ^+ cells. Furthermore, they found that NF-κB activity and its dependent proteins, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), were lower in bystander ρ^0 cells when compared with their ρ^+ counterparts. These results indicate that mitochondria play an important role in the regulation of radiation-induced bystander effects and that mitochondria-dependent NF-κB/iNOS/NO and NF-κB/COX-2/prostaglandin-E2 (PGE2) signaling pathways are important to the process. Additional experiments were performed using the Track Segment Facility to irradiate several cell lines plated on "strip" dishes (described above) with ^{4}He ions to identify the possible mechanism(s) of the radiation-induced bystander effect. Human-hamster hybrid (A_L) cells were examined for PKC expression (direct evidence) or PKC inhibitors were used (indirect evidence) to determine the involvement of the PKC pathway. Normal human lung fibroblasts/skin fibroblasts, with (ρ^+) or without (ρ^0) mitochondrial function, were examined to identify the signaling pathways. Prostate cancer cells were examined to determine if radiation can induce the bystander effect and to identify the possible mechanism.

Yigal Horowitz of Ben Gurion University of the Negev and Atara Horowitz of the Nuclear Research Center, Beersheva, Israel have resumed their investigation of the use of thermoluminescent dosimeters (TLDs) as nanodosimeters (Exp. 112). Recent work at Ben Gurion University has demonstrated that the major thermoluminescent dosimetry glow peak (peak 5) in the LiF:Mg,Ti (TLD-100) system is composed of at least three sub-entities of different sensitivities to ionization density. Glow peak 5a (at a temperature ~10 K less that of the main peak) is more intense following heavy charged particle irradiation and is believed to arise from localized (geminate) recombination in a molecular complex of ~ 20Å dimensions. The relative intensity of peak 5a to peak 5 is therefore a measure of ionization density, which has been proposed as a solid-state nanodosimeter that can, to a certain extent, mimic the ionization density dependence of radiation damage in DNA. TLD chips, both "normally cooled" and "slow cooled", were irradiated with 10^9 particles/cm^2 of low- and high-energy protons, deuterons and helium ions, as well as 0.2 and 14 MeV neutrons and 60 and 250 kV X rays. Results will be compared with-full track Monte Carlo calculations using an adaptation of the program FLUKA to derive radial dose deposition profiles in nanometer-sized volumes for low energy protons and alpha particles. Initial analysis (peak-height and glow peak width) has revealed a rich degree of intriguing and previously unobserved characteristics. These will be analyzed using computerized glow curve deconvolution.

A group led by Elena Aprile of the Columbia Astrophysics Laboratory of Columbia University resumed their calibration of a liquid xenon proportional counter (Exp. 123) to be used to detect weakly interacting massive particles (WIMPs). These are heavy neutral particles that only interact weakly with matter and may be the "dark matter" that will make up the "missing" mass in the universe. Electron ion pairs formed by ionization recombine (in a proportion that is a function of the electric field applied) and eventually produce scintillation photons as well. As most of the energy lost by a recoiling xenon nucleus will be converted into atomic motion instead of transferred to electrons through ionization, the xenon scintillation signal will be smaller than that of an electron recoil of the same energy. The scintillation efficiency of the nuclear recoils can be measured by first calibrating the detector with a monoenergetic gamma source and then producing the same energy xenon recoils. Low-energy neutrons scattered at a fixed angle by the xenon nuclei were detected as a function of time after pulses produced by the neutrons in the xenon detector. Since the initial neutron energy is known, the energy imparted to the xenon nucleus can

be calculated. Several different neutron energies and angles were used to try to calibrate the detector for the smallest pulses. Their measurements at RARAF have allowed them to make the most sensitive measurements for the search for WIMPs (limit on particle mass and cross section) in the world and their experiment was featured in an article in Nature in 2007. This group ran their experiments around the clock, sometimes for an entire weekend.

A group led by Sally Amundson of the CRR continued two types of experiments concerning radiation-induced gene expression profiles in primary human fibroblast and epithelial cell lines using cDNA microarray hybridization and other methods. One experiment, performed by Shanaz Ghandhi and Jaeyong Ahn, involved use of the track segment irradiation for comparison of gene expression responses to direct and bystander irradiation (Exp. 133). Human fibroblast cells (IMR90) and epithelial cells: (HBEC-3KT and SAEC) were plated on "strip" dishes (see above) for direct-contact bystander irradiations. The cells were irradiated with 0.5Gy of 125 keV/μm ^{4}He ions and assayed for micro-nucleus formation. The timing of micronucleus and gene expression assays is critically important and the time windows in which to perform these assays have been optimized. Using the fibroblast model, they now have identified potential genes and pathways involved in the bystander effect that are being validated by real-time PCR. They currently are working on identifying potential genes of interest in epithelial cells from the microarray studies which, in combination with fibroblast studies, will further our knowledge of bystander responses induced by radiation.

The second experiment (Exp. 136), performed in collaboration with Alexandre Mezentsev and Sunirmal Paul of the CRR, involved irradiation of artificial human tissue samples using the Track Segment Facility. Tissue model, Epi-200 (Mat-Tek, Ashland, MA) precisely imitates the structure of the epidermis. It is composed of ~20 layers of cells, each layer representing keratinocytes at a certain step of their terminal differentiation program. The tissues were irradiated with protons having an initial LET of ~10 keV/μm, either over the entire tissue surface or in a narrow line (~25 μm) across the diameter using the slit masks described above. After 48h, the tissues were removed from the culture insert and cut into narrow slices (200-400 mm) parallel to the irradiation line. Interest is focused on the lowest cell layer attached to the supporting membrane, since this is the only layer where cells divide. Irradiation of these cells allows observation of their performance and that of their descendents: undifferentiated keratinocytes (epidermal stem cells) that remain attached to the membrane, *via* analysis of their proliferation and mutagenesis, and differentiating cells, via study of their survival rate and metabolism. The experiments are based on three approaches: micronucleus assay, immunohistochemistry and microarray analysis. The first approach assesses the mutagenic potential of irradiation, answering the question whether irradiation interferes with cell division, and measures changes in cell proliferation rate. The second–approach detects and quantifies apoptosis in bystander and directly irradiated cells. The third approach identifies genes involved in the initiation, transition and termination of bystander effects. These analyses require multiple samples from the same tissue; often their size does not exceed a few mm^2. This required development of two new protocols: one for the purification of total RNA, another for the isolation of keratinocytes from 250 μm-wide tissue strips (one strip is ~3% of the tissue area). While the data analysis is still in progress, preliminary results suggest the existence of bystander effects after proton irradiation (0.10 and 2.5Gy). These effects appeared as higher frequencies of micronuclei, increased apoptotic rates and differential gene expression in bystander cells compared to non-irradiated controls.

Edouard Azzam, Janine Santos and Olga Kovalenko of the New Jersey School of Medicine and Dentistry continued to investigate whether mitochondrial DNA (mtDNA) damage by itself can trigger apoptosis in hTERT cells (Exp. 138). Parental cells carrying wild type or a nuclear-only hTERT mutant are irradiated either in the nucleus or the cytoplasm using the Microbeam Facility, allowed to recover for approximately 24h, and stained with YOPRO-1 in order to score the percentage of apoptotic cells. Using the same analysis procedure, they are also investigating radiation-induced bystander effects under conditions wherein a small fraction of cells in the exposed population is targeted through the nucleus or cytoplasm by one or more ^{4}He ions.

Ling Han of the Second Military Medical University, Shanghai, China, in collaboration with Dr. Tom Hei of the CRR, began an experiment to determine the expression, injury and signal transduction of the FHIT (Fragile Histidine Triad) gene (Exp. 140). The project is a combination of cell irradiation techniques, radiation biology and molecular biology. The Microbeam Facility is used to irradiate single cells in the nucleus, the cytoplasm or the culture medium. In other irradiations, only a fraction of the cells are irradiated and the co-cultured unirradiated (bystander) cells are examined. FHIT gene function is studied at different stages over 50 generations after irradiation and the role FHIT plays in cell transformation is examined in any transformed cells detected. They will explore effective ways to protect FHIT against radiation-induced injury as well as methods to select anti-irradiation drugs. They intend to produce databases for further study on mechanisms of microbeam low-dose biological effect and tumor radiotherapy. Newly established methods of drug selection will help to explore the mechanisms of anti-irradiation drugs as well as find new radiation-protective drugs.

Development of Facilities

This year our development effort continued on a number of extensions of our facilities:

- Development of focused accelerator microbeams
- Non-scattering particle detector
- Advanced imaging systems
- Targeting of cells
- Focused X-ray microbeam
- New laboratory space

Development of focused accelerator microbeams

The first quadrupole triplet lens (Fig. 2), installed in

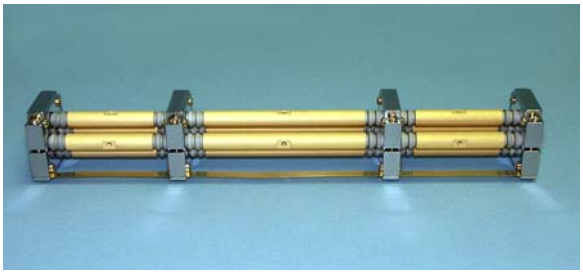

Fig. 2. The electrostatic triplet lens. The lengths of the sections are related to the electric field strengths required for focusing so that all voltages are similar in magnitude. Two of these lenses one meter apart make up the compound lens.

2003, has continued to operate very reliably. It has proven to be quite robust, surviving vacuum excursions caused by the occasional breakage of the ion beam exit window. An electrostatic phase space "sweeper" installed in 2006 just above the 90° bending magnet enabled us to focus a 6 MeV ^{4}He beam down to a diameter of 2μm. By varying the voltages on the 4 electrodes of the "sweeper", the beam is continually steered in a non-repetitive way and the object appears to the focusing system to be an isotropic source.

Two additional quadrupole triplets have been constructed and assembled into a single alignment tube as a compound lens in our machine shop by Gary Johnson. This compound lens has been installed in the beam line in place of the single lens. The lens alignment and focusing voltages are in the process of being adjusted to obtain a sub-micron beam spot diameter. At present the beam spot diameter is 1.3μm.

In order to test the alignment of the two triplet lenses, steering coils have been added in the space between the two triplets. This not only allows the beam to be steered from one triplet to the other but, by using two sets of coils with opposite fields, the beam can be displaced without changing the angle at which it enters the second lens. The current in the coils can be used to calculate in which direction and how far the bottom of the upper lens needs to be moved to align it with the lower lens.

After using this sub-micron beam for biological irradiations for a suitable period, the testing process will be repeated with a second compound triplet lens so that we will eventually have two complete compound lenses, one of which will be used as a spare.

A second microbeam using a compound quadrupole triplet lens made from commercially available precision permanent magnets (Fig. 3) was reassembled after the construction of the 3rd floor. Because the magnet strengths are essentially fixed, only a single energy (5.3 MeV) proton or ^{4}He ion can be focused. The pair of quadrupole triplets is similar to the one designed for the sub-micron microbeam, the major difference being that it uses magnetic rather than electrostatic lenses. This system was originally designed to focus alpha particles from a ^{210}Po source for use during the dismantling of the Van de Graaff and the installation of the Singletron. Using a charged particle beam from the accelerator provides us with a much greater flux and a smaller beam spot size because of reduced energy spread. The endstation for our original collimated microbeam was moved from the 2nd floor to the new microbeam lab on the 3rd floor because additional

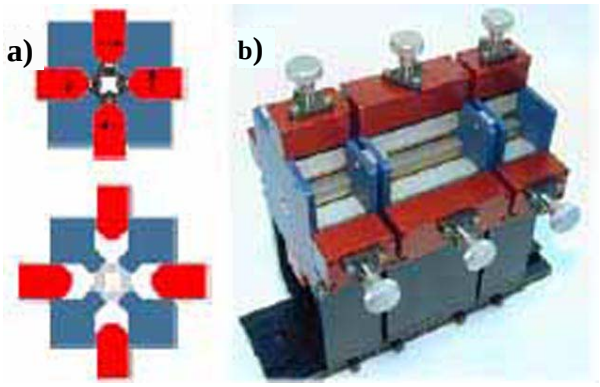

Fig. 3. Permanent magnet quadrupole triplet: a) Adjustment of the field by moving the magnets in and out. b) Triplet.

room for the lens structure is required between the final bending magnet and the focal point. After realignment of the system, and **without adjusting the magnets**, a beam spot size of 20μm was measured, demonstrating the robustness of this design.

A magnetic steering coil "sweeper" is used to produce a beam in which the particle location and direction are not coupled, as is done for the electrostatically focused microbeam, allowing the beam to be focused to a smaller diameter and increasing the flux at the endstation. The "sweeper" used here is based on the same split-coil used for the point and shoot system described below (Targeting Systems).

The lenses have been optically aligned and the quadrupole magnet strengths used to focus the beam are being adjusted using micrometric screws to retract and extend the individual magnets of each quadrupole. After installation of the phase space "sweeper" and a smaller object aperture (0.5mm), a beam of 5.3 MeV ^{4}He has been focused into a spot 8μm in diameter (a demagnification of x60, compared to the theoretically attainable x100). A miniature Hall probe has been purchased and will be used to map the magnetic fields of the lenses to look for aberrations and determine the octupole moment of the lenses, both of which would interfere with focusing.

This system will be used primarily for irradiations when the electrostatic system is unavailable because of development or repair and is presently being used to test the point and shoot system.

Non-scattering particle detector

Currently the RARAF microbeam irradiator delivers a precise number of particles to thin samples by counting the particles traversing them, using an ionization chamber placed immediately above the cells. To irradiate thick samples, such as model tissue systems or oocytes, to use particles with very short ranges, such as the heavy ions from the laser ion source, and to allow irradiation of cell monolayers without removing the culture medium, a completely non-scattering particle detector is necessary upstream of the samples. A novel particle detector has been designed on the basis of a long series of capacitive pick-up cells coupled together into a delay line. The Lumped Delay Line Detector (LD^2) consists of 250 silver cylinders, each 3 mm long with

a 2.2 mm inside diameter, connected by inductors and capacitively coupled to ground. The cylinders are glued to a semi-cylindrical tube of dielectric material 1 m long for mechanical support. The dielectric has a semi-cylindrical metal tube around it that can be rotated about one edge to adjust the capacitance. If the individual capacitance is set such that the propagation velocity of the pulse equals the projectile velocity, the pulses capacitively induced in all segments by the passage of a single charged particle will add coherently, resulting in a fast electron pulse at each end of the delay line that is 125 times larger than the charge induced on a single cylinder. This easily detectable charge of at least 125 electrons will be amplified to provide the detection pulse for the particle counter. After two prototype LD^2 detectors (1/6 length) were tested, the full-length detector has been constructed. Electrical measurements indicate that the propagation velocity in the delay line corresponds well to the calculated value. The detector will be placed in a horizontal beam line for testing with charged particle beams. After testing, the detector will be mounted between the two electrostatic lenses in the microbeam and become the standard detector for all microbeam irradiations.

Advanced imaging systems

Development continued on new imaging techniques to view cells without using stain and to obtain three-dimensional images of unstained cells.

The immersion-based Mirau interferometric (IMI) objective has been designed to function as an immersion lens with standard interferometric techniques by acquiring images at four positions with sub-wavelength separations using the vertical motion of the microbeam stage. Preliminary results imaging 10 μm D polystyroid beads in air were sufficiently encouraging to warrant the effort to design the new objective. In 2006, a commercial Mirau objective was modified by filling part of the light path with water so that it could be used as a water immersion lens. Tests confirmed that the lens can provide interference fringes with sufficient contrast to perform the biological experiments. A custom Mirau objective has been constructed in our shop and several beamsplitters of different reflectivity (5-85%) have been obtained. These have been combined with spot mirrors into separate modules so that they can easily be interchanged in the lens. Vertical motions due to vibrations in the building reduce the image quality. A Fourier technique is being investigated to remove the effects of the vibrations.

A multi-photon microscope has been developed for the single-cell single-particle Microbeam Facility to detect and observe the short-term molecular kinetics of radiation response in living cells and to permit imaging in thick targets, such as tissue samples. Two photons delivered closely together in space and time can act as a photon with half the wavelength (twice the energy). The longer wavelength of the actual light beam allows better penetration into the sample while still being able to excite the fluorophor. The multi-photon capability has been integrated into the Nikon Eclipse E600-FN research fluorescence microscope of the microbeam irradiation system and will provide three-dimensional imaging. A Chameleon (Coherent Inc.) tunable

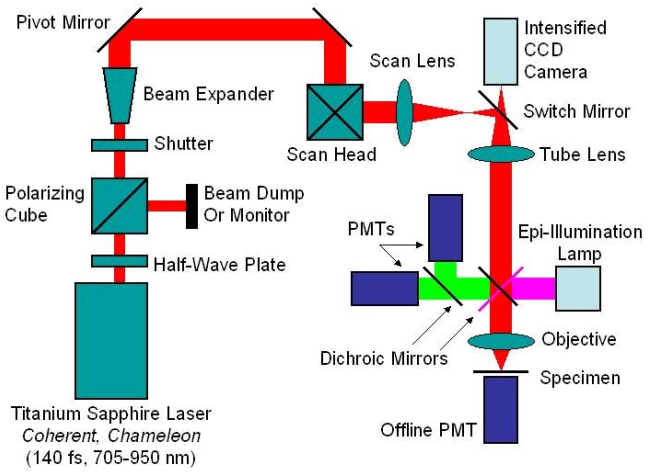

Fig. 4. Diagram of the multiphoton microscope optics path.

titanium sapphire laser (140 fs pulses at a 90 MHz repetition rate) is the source for the multi-photon excitation. The scan head incorporates commercial scanners and a scan lens then focuses the laser beam to a point at an image plane of the microscope (a CCD camera is also placed at such an image plane for fluorescent microscopy). The incident laser beam enters the microscope through the side of the trinocular tube of the microscope (Fig. 4). A switch mirror allows us to choose between multiphoton microscopy and standard fluorescence microscopy. The scanned laser beam establishes an optical section within the specimen, where multi-photon absorption preferentially occurs. Wavelengths available from the laser can penetrate to depths of about 100 microns in a biological sample by varying the Z-position of the specimen stage. Light emitted from the specimen is selectively deflected by a series of dichroic mirrors to an array of photomultiplier tubes (PMTs). To control the multi-photon microscope, we are adopting the design and software of Karel Svoboda, Cold Spring Harbor.

Initial two-color images of stained cells are excellent. Presently only one PMT can be mounted on the system. A housing that will enable us to use two PMTs and therefore obtain simultaneous images from two fluorophors, is being constructed.

Two PMTs will allow the investigation of fluorescence resonance energy transfer (FRET). Molecules labeled with two fluors normally widely separated can change their conformation by phosphorylation, positioning the two fluors near each other. In this close proximity, the emission from one fluor can excite the other, changing the ratio of emissions from the two fluors. A measure of the amount of phosphorylation in the sample can be determined.

Targeting of cells

During irradiation, cells to be exposed are moved to the beam position using a combination of a high-resolution three-axis piezo-electric inner stage (Mad City Labs, Madison, WI) with a limited range and a motor-driven outer stage with a larger range but poorer accuracy. When a collimated microbeam was being used, this is a necessary but relatively time-consuming method to position cells for irradiation. Unlike a collimated microbeam, a focused microbeam is not

restricted to a single location on the exit window and therefore can be deflected to any position in the field of view of the microscope used to observe the cells during irradiation. Moving the beam to the cell position magnetically or electrostatically can be performed much faster than moving the stage.

We are developing a "point and shoot" targeting system for microbeam irradiation based on wide-field magnetic split-coil deflector system from Technisches Büro Fischer (Ober Ramstadt, Germany). A dual deflection amplifier, optimally matched to these coils, has been purchased from the same company to drive the coil. This system has been used for the microbeam facility at Gesellschat für Schwerionenforschung (GSI), Darmstadt, Germany. One coil has been operated with this amplifier as a beam "sweeper" for the PMM, but the amplifier has been replaced by one designed and built in-house, freeing it for its original purpose. A short section of beam line has been constructed around which the coil has been placed. The coil assembly is mounted just below the upper quadrupole triplet on the PMM, where it is being tested and the deflection calibrated against the coil current.

Focused X-ray microbeam

There are considerable benefits in using soft X-ray microbeams for both mechanistic and risk estimation endpoints. The higher spatial resolution achievable with modern state-of-the-art X-ray optics elements combined with the localized damage produced by the absorption of low-energy photons (~1-5 keV) represents a unique tool to investigate the radio-sensitivity of sub-cellular and eventually sub-nuclear targets. Moreover, as these X rays do not suffer from scattering, by using higher energy X rays (~5 keV) it is possible to irradiate with sub-micron precision individual cells and/or part of them up to a few hundred microns deep inside a tissue sample in order to investigate the relevance of effects such as the bystander effect in 3D structured cell systems.

We have investigated expanding the microbeam to include characteristic K_α X rays generated by proton-induced emission (PIXE) from Ti (4.5 keV). The use of higher energies is not feasible due to Compton scattering effects; we are limited to X-ray energies where the predominant mode of interaction is photoelectron absorption. Charged particle beams can generate nearly monochromatic X rays because, unlike electrons, they have a very low bremsstrahlung yield.

At the suggestion of one of the members of our Advisory Committee, we have changed from a transmission design, in which the X rays used are emitted in the direction of the proton beam, to a reflection design, in which the X rays used are emitted at 90° to the proton beam direction. This eliminates several problems inherent in the previous design. The system will be mounted on its own horizontal beam line on the 1st floor of the Facility and the X-ray beam will be oriented vertically, so that the geometry of the microscope and stage will be the same as for our other microbeam systems.

The new target structure consists of a round titanium plug with an angled surface embedded in a copper cooling block. A small X-ray source (~20 μm D) will be produced by bombarding the Ti target with high-energy protons using the quadrupole quadruplet lens used for our first focused microbeam, reducing the requirements on the subsequent X-ray focusing system.

A zone plate will be used to focus the X-ray source to a beam spot 1-2 μm in diameter. The proposed zone plate will have a radius of only 120μm, an outmost zone width of 50 nm and a demagnification factor of ~11. The final expected dose rates to the sample, based on ANSYS simulations, are 1 to 6 mGy/s.

The main elements of the system have been manufactured in our machine shop and the zone plates are scheduled to be purchased this year.

New laboratory space

Because of a large research and development grant received by David Brenner and Gerhard Randers-Pehrson from the National Institute of Allergy and Infectious Diseases (NIAID), the Trustees of Columbia University in 2006 contributed the funds required to build over 2000 square feet of new laboratory and office space on the third floor of the Facility. Construction was completed in December, 2006. The main lab, which comprises over half the area, has been equipped with a class II biological flow hood, incubator, refrigerators, a freezer, centrifuges and other equipment and is in regular use. The office area has had as many as 5 people occupying the desks.

The other three laboratories are also in use. The PMM facility has been reconstructed in the new microbeam lab. The microscope lab has 3 fluorescent microscopes, including a new system for doing mFISH analysis, and a NIAID pilot project is being conducted in the physics lab.

Singletron Utilization and Operation

Table 2 summarizes accelerator usage for the past year. The Singletron is started at 7:30 AM on most days from September through June and by 9 am the rest of the time. It is often run into the evening, and frequently on weekends for experiments, development and repair. This has resulted in a total use that exceeds the nominal accelerator availability of one 8-hour shift per weekday (~250 shifts per year).

Use of the accelerator for radiobiology and associated dosimetry was only about 2/3 that of last year (which had the highest level of use since RARAF has been at Nevis Labs), but was only slightly below the average for the last 5 years. This was due, in part, to an 80% increase in on-line devel-

Table 2. Accelerator Use, January–December 2007
Usage of Normally Scheduled Days

Radiobiology and associated dosimetry	30%
Radiological physics and chemistry	19%
On-line facility development and testing	46%
Safety system	2%
Accelerator-related repairs/maintenance	7%
Other repairs and maintenance	3%
Off-line facility development	25%

opment. About 42% of the use for all experiments was for microbeam irradiations and 24% for track segment irradiations. The Microbeam Facility continues to be in great demand because it enables selective irradiation of individual cell nuclei or cytoplasm. In addition, because of the relatively low number of cells that can be irradiated in a day, microbeam experiments usually require significantly more beam time than broad beam (track segment) irradiations to obtain sufficient biological material, especially for low probability events such as mutation and bystander effects.

The Track Segment Facility is being used very efficiently, reducing the amount of accelerator time required to satisfy user demand. Because the irradiation times for samples are often 10 seconds or less, multiple users, as many as 5, are run on a single shift, sometimes using different LETs and even different types of ions in the same day.

Radiological physics utilization of the accelerator increased by about 60% this past year. Three physics experiments made use of the accelerator (Exp. 82, 112 and 123), more than usual. The experiment for Astrophysics (Exp. 123) alone used about 24% of the experiment time mainly at night and on weekends.

Approximately 41% of the experiment time was used for experiments proposed by outside users, about twice what was used last year and 1/3 more than the average for the last five years. Again, much of this increase is due to the Aprile group (Exp. 123).

Use of the accelerator for online development increased by about 80% over last year and was slightly higher than the average use for the past 5 years. In addition to beam tests and development of the electrostatically focused microbeam, considerable effort was expended on minimizing the beam spot diameter for the permanent magnet microbeam (PMM).

Singletron maintenance and repair time was only 2/3 that of last year, the lowest it has been in about 10 years. The new Singletron accelerator has operated relatively reliably for 26 months. Until October, there were only two accelerator openings in 2007, totaling less than 6 days: one at the end of May to replace the ion source bottle, which had become dirty after 7 months of service, and one in the beginning of July to replace the RF tubes. We expect the source bottle will have to be changed every 9-12 months, depending on accelerator use, but we haven't had enough experience to determine an average lifetime. In October a problem developed in the generating voltmeter (GVM), which is used to measure the terminal voltage on the accelerator. The output became unstable, causing the terminal voltage to become unstable through the voltage control system. The problem was believed to be the bearings in the GVM motor. In the process of replacing the bearings, an electrical connection on the GVM detection plate was damaged. After installing the GVM, we found the repair had failed. Two more attempts to make the repair were made before we installed a temporary replacement GVM loaned from by the accelerator manufacturer, High Voltage Engineering Europa (HVEE). These openings totaled over 8 days of effort. The damaged GVM was sent to HVEE for repair (it has just returned at the beginning of February, 2008). The replacement GVM also was unstable (~30 kV), even with no terminal voltage. It was discovered that the charging supply is so stable that we are able to run without terminal voltage regulation by setting the charging current to obtain the desired terminal voltage, which is how the accelerator has been running for almost 4 months.

The drift in the terminal voltage as the accelerator warms up during the day, which was discovered in 2006, has continued to be an annoyance. Because the beam energy acceptance is so narrow for the electrostatic microbeam and the PMM, beam intensity decreases rapidly as the terminal potential changes by as little as a kilovolt. A remote computer terminal was installed in the Microbeam II lab to allow the accelerator terminal voltage to be controlled from both the console and the lab until a system to maintain the temperature of the GVM can be designed and installed. Another remote computer terminal will have to be installed for the PMM.

Training

The Small Group Apprenticeship Program continued for the fourth year. Five students from Stuyvesant High School in Manhattan spent at least two half-days each week for six weeks during the summer working on projects in biology (1) or physics (4). Stuyvesant is a high school specializing in science that is open to students throughout New York City by competitive admission. The students gave professional PowerPoint presentations to our group at the end of the program. Below is a list of the titles of the work presented followed by the name of the student and the name of his or her mentor:

1. Adaptive Responses in Irradiated Cells - Farhan Nuruzzaman (Brian Ponnaiya)
2. Under-dish Detector for the Microbeam at Columbia University - Chaitanya Medicherla (Guy Garty)
3. Oxygen/Nitrogen Resonance Explosives Detector - Li Ang Zhang (Yanping Xu)
4. Design of the RARAF X-Ray Microbeam - Dawood Din (Andrew Harken)
5. Multiphoton Microscope Development - Benjamin Lerner (Alan Bigelow)

Several of the previous students have been co-authors of journal articles, including one in the prestigious Proceedings of the National Academy of Science (PNAS).

Andrei Popescu, a student at Ossining High School in Westchester County, has worked with Brian Ponnaiya for almost a year. He is studying DNA breakage and micronucleus formation in mouse cells after X-ray irradiation.

Personnel

The Director of RARAF is Dr. David Brenner, now also the Director of the Center for Radiological Research. The accelerator facility is operated by Mr. Stephen Marino, the manager, and Dr. Gerhard Randers-Pehrson, the Associate Director of RARAF.

Dr. Charles Geard, the former Associate Director of the CRR and the Senior Biologist for the P41 grant that is the major support for RARAF, continues to spend most of each day at RARAF. In addition to his own research, he collaborates with some of the outside users on experiments using

the single-particle Microbeam Facility.

Dr. Alan Bigelow, an Associate Research Scientist, is developing a multiphoton microscopy system using a fast laser.

Dr. Guy Garty, an Associate Research Scientist, is developing an inductive detector (LD^2) for single ions and the permanent magnet microbeam (PMM). He spends about half his time working on the National Institute of Allergy and Infectious Diseases (NIAID) project, for which he is the project manager.

Sasha Lyulko, a graduate student in the Physics Department at Columbia, is involved in developing methods to image cells without stain and spends about half her time working on the NIAID project.

Dr. Andrew Harken, a Postdoctoral Fellow, is developing an X-ray microbeam and working with Guy Garty on the PMM.

Dr. Yanping Xu, a Postdoctoral Fellow, has been working on the NIAID project, developing a method for determination of the number of lymphocytes in blood samples using light absorption.

Several biologists from the Center for Radiological Research are stationed at the facility in order to perform experiments:

- Dr. Brian Ponnaiya is an Associate Research Scientist performing experiments using the Track Segment and Microbeam irradiation facilities.
- Ms. Gloria Jenkins, a biology technician, performed experiments on the Microbeam Facility for Dr. Geard. Gloria retired in May after 34 years with Columbia University and 17 with the Center for Radiological Research.
- Dr. Alexandre Mezentsev, an Associate Research Scientist, is working with cultured tissue systems and spends some of his time at RARAF.
- Dr. Helen Turner, an Associate Research Scientist, is working on the NIAID project and spends at least one day per week at RARAF.

At the end of March Yigal Horowitz from Ben Gurion University of the Negev and Atara Horowitz from the Nuclear Research Center, Beersheva in Israel began a one-year sabbatical at RARAF. Their project involved the characterization of 'slow-cooled' LiF:Mg,Ti (TLD-100) using neutrons and charged particles. They returned to Israel in November for personal reasons.

Kenichi Tanaka, a Staff Associate, arrived in August from Hiroshima University, Japan for a one-year visit and has been working with Gerhard Randers-Pehrson on the detection of explosives. He is terminating his visit in March in order to accept a position at the University of Hokkaido.

Recent Publications of Work Performed at RARAF

1. Hei TK. Radiation-Induced Bystander Effects: Mechanisms and Implication for Low-Dose Radiation Risk Assessment. Radiat. Res. **167**: 347-8, 2007.
2. Hei TK, Zhou H, Ivanov VN, Hong M, Lieberman HB, Brenner DJ, Amundson SA and Geard CR. Mechanism of radiation induced bystander effects: a unifying model. *J. Pharmacy and Pharmacology* (In press).
3. Hei TK, Zhou HZ, Lien YC and Zhao YL. Mechanism of radiation carcinogenesis: BigH3, COX-2 and beyond. *Int. Congress Series*, **1299**: 114-20, 2007.
4. Ivanov VN, Zhou H and Hei TK. Sequential treatment by ionizing radiation and sodium arsenite dramatically accelerates TRAIL-mediated apoptosis of human melanoma cells. *Cancer Res* **67**:5397-407, 2007.
5. Miller AC, Stewart M, Rivas R, Marino S, Randers-Pehrson G and Shi L. Observation of radiation-specific damage in cells exposed to depleted uranium: *hprt* gene mutation frequency. Radiat. Meas. **42**: 1029-32, 2007.
6. Nikolaev A, Oks EM, Savkin K, Yushkov GYu, Brenner DJ, Johnson G, Randers-Pehrson G, Brown IG and MacGill RA. Surface resistivity tailoring of ceramic insulators for an ion microprobe application. *Surface & Coatings Technology* **201**:8120-8122, 2007.
7. Ponnaiya B, Jenkins-Baker G, Randers-Pherson G and Geard CR. Quantifying a bystander response following microbeam irradiation using single-cell RT-PCR analyses. *Exp Hematol* **35**:64-8, 2007.
8. Sedelnikova OA, Nakamura A, Kovalchuk O, Koturbash I, Mitchell SA, Marino SA, Brenner DJ and Bonner WM. DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models. *Cancer Res* **67**:4295-302, 2007.
9. Sykora GJ, Akselrod MS, Salasky M and Marino SA. Novel Al2O3: C,Mg Fluorescent Nuclear Track Detectors For Passive Neutron Dosimetry. *Radiat Prot Dosimetry*, NEUDOS10 Special Edition: 1-6, 2007.
10. Williams ES, Stap J, Essers J, Ponnaiya B, Luijsterburg MS, Krawczyk PM, Ullrich RL, Aten JA and Bailey SM. DNA double-strand breaks are not sufficient to initiate recruitment of TRF2. *Nat Genet* **39**:696-8; author reply 8-9, 2007.
11. Zhou H, Ivanov VN, Lien YC, Davidson M and Hei TK. Mitochondrial function and nuclear factor-kappaB-mediated signaling in radiation-induced bystander effects. *Cancer Res* **68**:2233-40, 2008. ■